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P444/0001
GSL/GOSO:gl**IN THE UNITED STATES PATENT & TRADEMARK OFFICE**

Inventor(s): HUANG, Dong; QI, Dong Feng
Title: NOVEL AGLYCON DAMMARANE SAPOGENINS, THEIR USE AS
ANTICANCER AGENTS, AND A PROCESS FOR PRODUCING SAME
SERIAL No.: 09/910887
Filed: 24 July 2001
Examiner: Qazi, Sabiha Naim Art Unit: 1616
Date: March 4, 2005

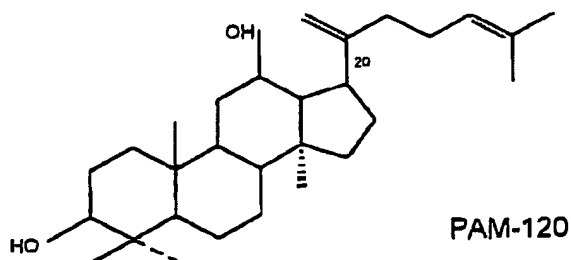
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Commissioner for Patents
P.O. Box 1450
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Dear Sir,

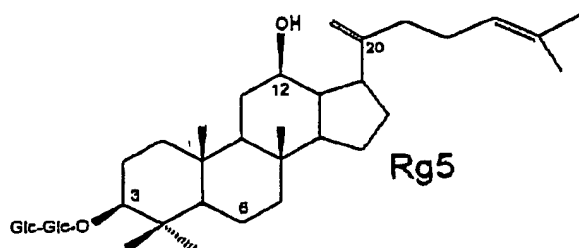
AFFIDAVIT UNDER RULE 1.132

I, Dong Huang, of 16788 102 Avenue, Surrey, British Columbia, Canada V4N 4X2, MAKE OATH
AND SAY AS FOLLOWS:

1. I have personal knowledge of the matters sworn to herein, except where the matters are stated to be based on information and belief, in which case I believe them to be true.
2. I am a co-inventor of the invention described and claimed in US Patent Application Serial No. 09/910887.
3. I hold a Bachelor of Science degree from the University of Beijing China.
4. I have over 20 years of experience in the fields of botany chemistry research and ginsenoside drug development.
5. I have conducted side-by-side experiments to compare the efficacy of the compounds PAM-120 and Rg5.
6. PAM-120 has the following chemical structure:



Rg5 has the following chemical structure:



7. I conducted experiments to compare the efficacy of PAM-120 and Rg5 against lung cancer cells in the following manner. Compounds PAM-120 and Rg5 were obtained from Pegasus Pharmaceuticals Group Inc. Human non-small-cell H460 lung cancer cells were seeded at 3×10^4 cells/well in a 96-well plate in DMEM medium supplemented with 10% fetal bovine serum and incubated at 37°C with 5% CO₂. The cells were treated with isolated ginsenoside PAM-120 and Rg5 at a fixed dose of 25 μ M. The cytotoxic effects of the compounds on the lung cancer cells were measured by determining the viability of the cells. Cell viability was measured using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay method (Denizot and Kang, J. Immunol. Meth. 89:271-277 (1986); Carmichael et al., Cancer Res. 47:936-942 (1987)) 24 hours following treatment. Cell viability was measured by determining the absorbency of stained cells. Non-viable cells have lower absorbency compared to viable cells. Table 1 shows the viability of H460 lung cancer cells in the presence of the compound PAM-120 and Rg5 at 25 μ M.

Compound (25 μ M)	Absorbency of stained cells ($M \pm SD$)	Viability (%)
Blank Control	0.352 \pm 0.062	100.00
PAM-120	0.218 \pm 0.043	61.93
Rg5	0.290 \pm 0.065	82.38

Table 1: Viability of H460 Lung Cancer Cells in the Presence of 25 μ M Pam-120 and Rg5.

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8. The results in Table 1 illustrate that H460 lung cancer cells are significantly less viable in the presence of PAM-120 than Rg5. Therefore PAM-120 has greater cytotoxic effects than Rg5.
9. I conducted experiments to compare the efficacy of PAM-120 and Rg5 against drug-resistant breast cancer cells in the following manner. Compounds PAM-120 and Rg5 were obtained from Pegasus Pharmaceuticals Group Inc. Human drug-resistant MCF7r breast cancer cells were seeded at 3×10^4 cells/well in a 96-well plate in DMEM medium supplemented with 10% fetal bovine serum and incubated at 37°C with 5% CO₂. The cells were treated with isolated ginsenoside PAM-120 and Rg5 at various concentrations. The IC₅₀s of the compound PAM-120 and Rg5 were determined using standard methods. IC₅₀ is the concentration of a compound needed to reduce the growth of a population of cells by 50%. The IC₅₀s of the compounds are shown in Table 2.

Compound	IC ₅₀ (µg/mL)
PAM-120	<10
Rg5	70 ± 5.4

Table 2: IC₅₀ Values of Compounds PAM-120 and Rg5 against MCF7r Breast Cancer Cells

10. The results in Table 2 illustrate that PAM-120 has a significantly lower IC₅₀ than Rg5. PAM-120 has an IC₅₀ over 7 times less than the IC₅₀ of Rg5. Therefore, PAM-120 is effective at inhibiting MCF7r breast cancer cells at a much lower concentration than Rg5.
11. I conducted experiments to compare the efficacy of PAM-120 and Rg5 against melanoma cells in the following manner. Compounds PAM-120 and Rg5 were obtained from Pegasus Pharmaceuticals Group Inc. Mouse B16 melanoma cells were seeded at 3×10^4 cells/well in a 96-well plate in DMEM medium supplemented with 10% fetal bovine serum and incubated at 37°C with 5% CO₂. The cells were treated with isolated ginsenoside PAM-120 and Rg5 at various concentrations. The IC₅₀s of the compound PAM-120 and Rg5 were determined using standard methods. IC₅₀ is the concentration of a compound needed to reduce the growth of a population of cells by 50%. The IC₅₀s of the compounds are shown in Table 3.


Compound	IC ₅₀ (µg/mL)
PAM-120	<10
Rg5	35 ± 3.9

Table 3: IC₅₀ Values of Compounds PAM-120 and Rg5 against B16 melanoma Cells

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12. The results in Table 3 illustrate that PAM-120 has a significantly lower IC50 than Rg5. PAM-120 has an IC50 value over 3 times less than the IC50 of Rg5. Therefore, PAM-120 is effective at inhibiting B16 melanoma cells at a significantly lower concentration than Rg5.

SWORN before me at the city of)
SURREY, in the province of British)
 Columbia, Canada this 7 day of March)
 2005)
 _____)
 A Notary Public in and for the)
 Province of British)
 Columbia, Canada. My Commission is for life)



 (Dong Huang)

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